MUCOCILIARY FUNCTION AS PROTECTIVE MECHANISM IN UPPER RESPIRATORY TRACT

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Three general aspects of mucociliary function will be considered: first, comparative anatomy and physiology; second, pathogenesis of certain infectious diseases in the upper respiratory tract; and third, the cell-virus relationships involved in infected organ cultures of respiratory mucosa.

Comparative Anatomy and Physiology

The general problem of comparing the anatomy of the upper respiratory tract of one animal species with that of another has been reviewed by several authors, notably Negus (14) and Proetz (18). These, however, have concentrated on problems directly related to respiratory physiology in man. There has been one general review of the situation in vertebrates (2). If one is dealing with a question of sinusitis, for example, in a species such as the dog, it is of obvious importance to determine whether the sinus involved in this infection is similar to that of man or whether it has a different drainage mechanism. As may be seen in Fig. 1, the maxillary sinus of a dog actually has a ventral and a dorsal opening and is more a recess than a sinus. The question of the degree to which the turbinates are involved with a particular infection obviously depends somewhat on the amount of surface epithelium. The complex turbinates of species such as the ferret or the cat (Fig. 2) can filter a large number of particles out of the air before it gets to the olfactory mucosa or down to the lungs. These are entirely different from the small blunt turbinates of man. The extent of the olfactory area itself may vary greatly among the different species. Its total area reflects the degree to which a species is dependent upon olfactory activity in its responses to the variety of environmental stimuli. There are several accessory organs in the upper respiratory tract, including the lateral nasal gland, which has been shown by Schmidt-Nielson (19) to be involved in the excretion of excess salt in a number of sea birds. However, this cannot be its only function, for, as seen in Fig. 3, large lateral nasal glands are present also in a variety of mammals and in in nonmarine birds. It is large in the common chicken which does not drink sea water and thus has no apparent need for excreting excess salt.

We mentioned the question of "true" sinuses. It is apparent that the question of whether olfactory epithelium extends, for instance, into a dog's or a cat's sinus depends upon one's definition of a sinus. Some of the larger ungulates have extensive "true" sinuses in the bony skull which far exceed in complexity those of man (Fig. 4). More information about such air spaces as they have evolved in individual species is clearly needed before generalizations about their function are justifiable.

Cilia and mucus have been associated in the animal kingdom for a long time both in the evolution of species and the development of individual species. The functional association of the two constitutes the ciliary-mucus blanket, formed when a film of mucus is excreted onto a ciliated surface which carries that blanket in a particular direction. This directional flow is a recognized characteristic of the upper and lower respiratory tracts. Lucas and Douglas (12), in their clear description of this motion observed on several domestic animals, showed that the blanket did not simply flow anteroposteriorly but flowed anteriorly from the cribriform area and either turned downward and backward into the pharynx or went into the vestibule. Much particulate debris is thus carried to the posterior region of the pharynx and so brought to a spot where it may either come in contact with a lympathic ring or with tonsillar tissue. Most of the blanket and its debris is presumably swallowed. In the primates no narially directed flow has been described.

Recently we have been examining the pattern of the mucous glands in the upper respiratory tracts of several birds (3*a*). This has been done by staining whole mounts of nasal organs by a modification (13) of the periodic acid-Schiff technique developed by Moe, which allows one to see

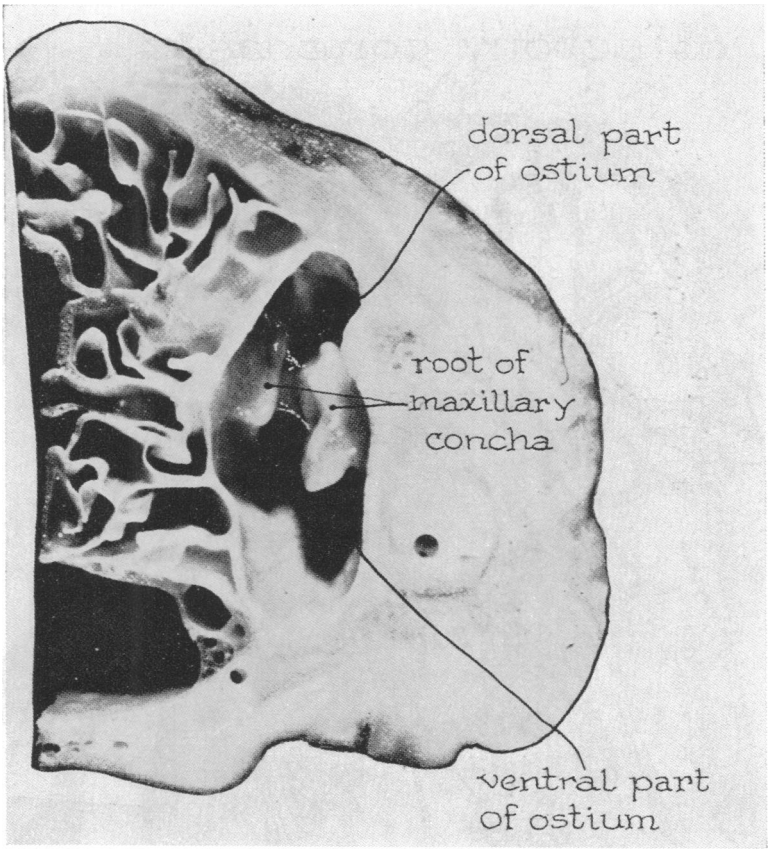

FIG. 1. *Postero-anterior view of right half of nasal chamber of beagle, showing division of maxillary sinus into two communicating chambers.*

the entire display of the mucous glands. To our surprise and pleasure a characteristic pattern in the surface distribution of these glands was demonstrated. The pattern was particularly evident on the septum. The natural question then was whether there was any relation between the gland alignment and the ciliary directional flow. As may be seen in Fig. 5, there is a direct relationship between the pattern of these mucous glands and the directional flow of this blanket. The flow of the blanket was determined by decapitating anesthetized birds and quickly making near-sagittal sections of the heads so that on the one side the septum was displayed and on the other side the turbinates and lateral wall structures. Then small droplets or thin films of India ink were placed on different areas of the mucosae and under a dissecting microscope the directional flow of this India ink was followed. The relationship has been established in several dozens of experiments.

Effect of Dehydration

Dalhamn (6) and others have shown that if the mucosa is dry, there is a rapid loss of activity of the cilia. The rate of movement of a particular particle along the tract is reduced. The effects of internal dehydration have not been studied. Recently, we stumbled onto this problem in the study of some sea gulls which were brought in from a neighboring island where they were caught. We discovered that the mucous blanket was not functioning adequately (3b). Indeed, when India ink was placed on this area, it completely failed to move. It was then recognized that these animals were acutely dehydrated. We have since shown that not only in the sea gull but also the common chicken and the mouse, lengthy deprivation of water may cause the blanket to be

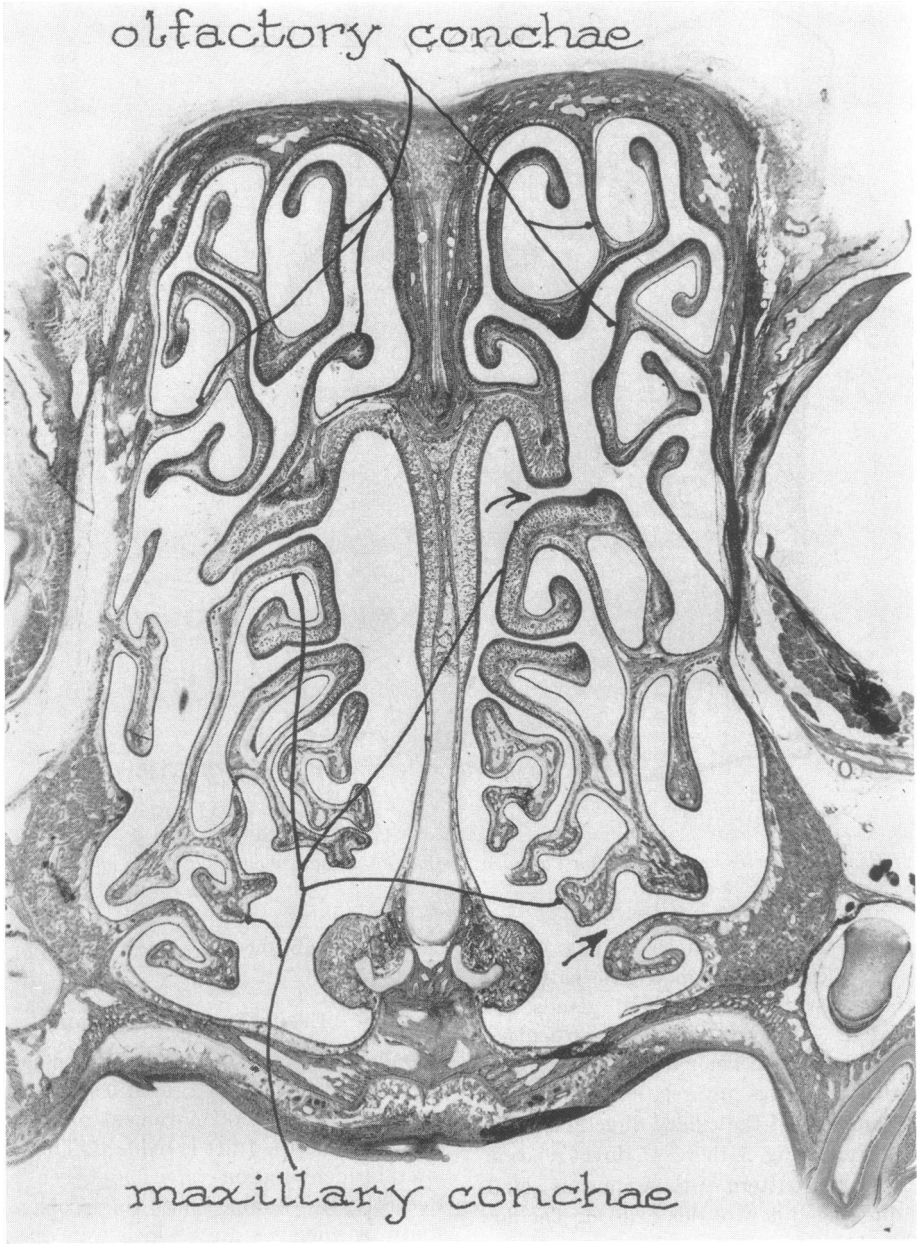

FIG. 2. *Antero-posterior view of low-power section of cat nasal chamber cut through maxillary and olfactory conchae, showing relationships of the two parts of ostium (arrows) to the functional conchae.*

markedly inhibited in its function, whereas the cilia do not seem to be equally affected. Whether this has an effect on susceptibility to infection we do not know, but if the primary function of the mucous blanket, which is to trap and dispose of particulate debris, is related in any way to the defensive mechanism, we should expect some effect. We mentioned earlier that the mucociliary function is a common mechanism throughout the invertebrate and vertebrate kingdoms. In the invertebrates it is a feeding mechanism whereby particles of certain sizes are caught and pulled into

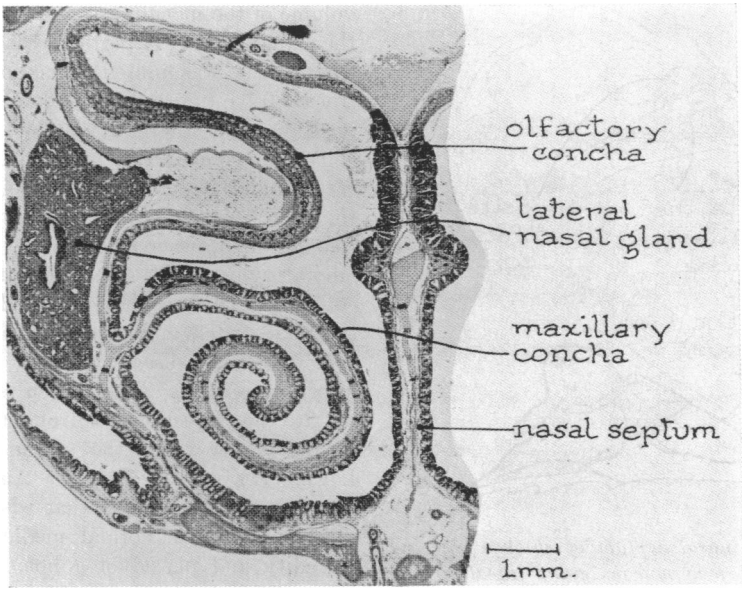

FIG. 3. *Right half of chicken nasal chamber cut through middle of olfactory concha. Low-power section showing size of lateral nasal gland body at this level.*

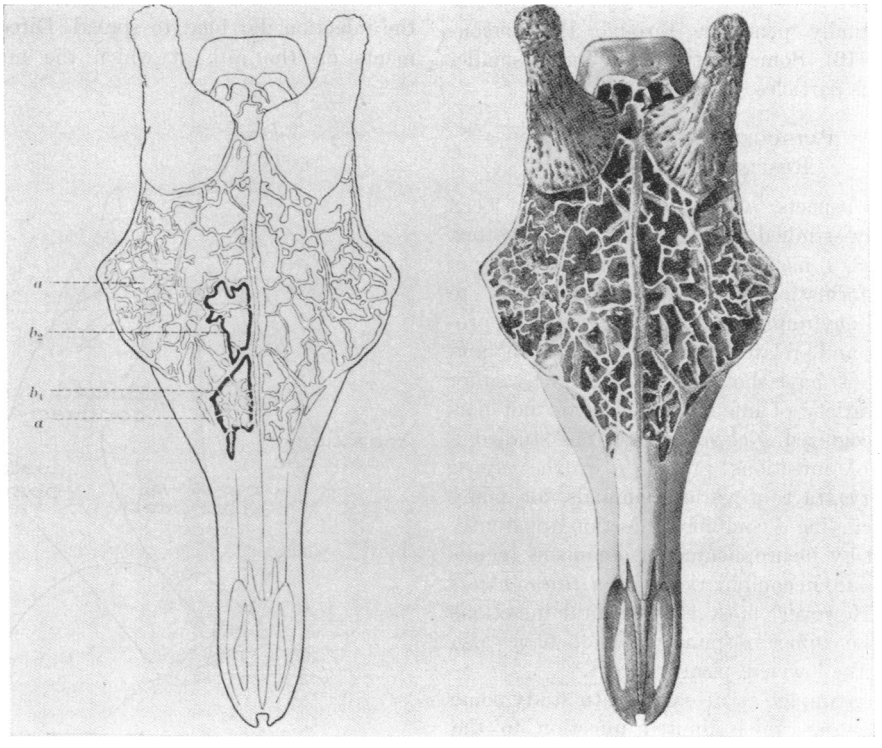

FIG. 4. *Giraffe skull with single opening to exterior. a, Sinus maxillaris; b_1, b_2, sinus fornicales. (From Paulli (16a).)*

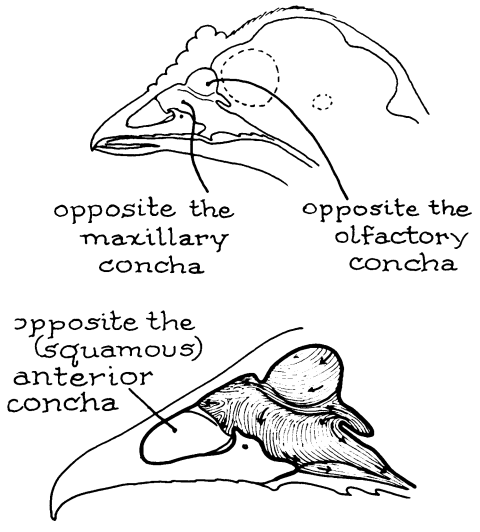

FIG. 5. *Right nasal septum of chicken showing direct relationship of mucous gland lineation and direction of mucous flow (arrows).*

the animal's mouth. In such filter feeding it has been shown that particles of a very small size may actually penetrate through the mucous blanket (10). Some of these are much smaller than virus particles.

Pathogenesis in Upper Respiratory Tract

Many aspects of pathogenesis have been separately studied by different investigators. Hilding (8, 9) has provided a tremendous body of basic information on mucosal responses to trauma, environmental change, surgical procedures, and related damage. Berberich and Kelemen (4) have shown that animals may suffer from a variety of infections which are not normally recognized. Nelson (15, 16) has studied a variety of infectious processes in the upper respiratory tract of various animals for many years, including a combined infection apparently produced by pleuropneumonia organisms (genus *Mycoplasma*) in combination with a *Haemophilus* species. However, most experimental infections within the upper respiratory tract have also involved the lower respiratory tract.

A few years ago in attempting to study some of the factors which limited infection to the upper respiratory tract, we found that a so-called mild (vaccine) strain of Newcastle disease virus produced such an infection in the chick if the volume of the inoculum was small. Regardless of the actual number of particles within this volume the infection was limited to the upper respiratory tract if 0.1 ml or less of the fluid was inoculated into an unanesthetized animal (5). Parenthetically, our comparative anatomical studies indicated that the simple upper respiratory tract of the chick is quite similar in general structure to that of man, whereas those of such common laboratory mammals as mice and ferrets are very complex. Figure 6 shows the relatively simple turbinate system and maxillary sinus of the chick.

If Newcastle disease virus is limited to the upper respiratory tract when small volumes initiate the infection, then one may study the factors which cause the spread of the virus. Lowry (11), working with Dr. Anna M. Baetjer a few years ago, showed that when chicks were taken from their normal medium of average humidity and placed in a hot, moist environment, then the few cases of upper respiratory tract infection which did spill over into the lower respiratory tract were eliminated. In contrast, if the chick was placed in a very dry hot medium, the infection did tend to spread. Direct experiments on the rate at which the mucociliary

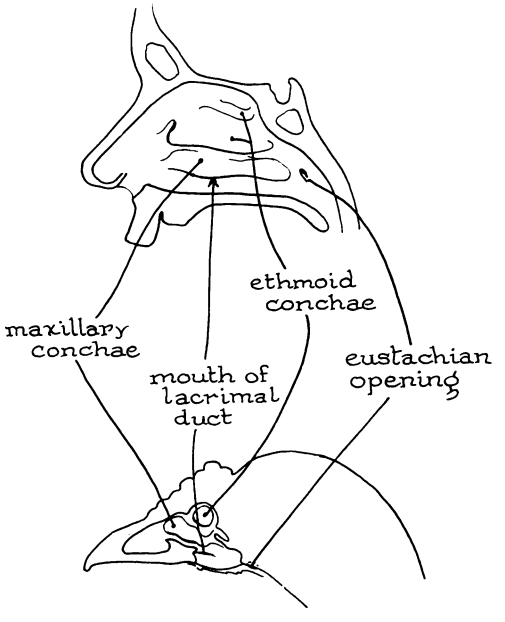

FIG. 6. *Right lateral wall structures of human and chicken nasal fossae showing similar simple conchae and relationships.*

blanket moved up the lower respiratory tract to the larynx showed that animals exposed to a hot dry medium had a much slower rate of transfer of particulate material. Perhaps the spread of virus from the upper to the lower respiratory tract occurs more readily when the mucociliary blanket is impaired by internal or external factors which prevent its optimal function.

Limitation of the experimental infection to the upper respiratory tract allows one to do certain other experiments. Experimental epidemiology of respiratory tract infections is practically unknown. Most experimental epidemiology has been done with either intestinal infections or with the pox viruses. We have shown that the upper respiratory model may be exploited in a similar way. If two chicks which have been infected with the mild strain, which is limited to the upper respiratory tract, are then introduced into a small flock, one may follow the spread of the infection through that flock. We have been carrying out such demonstrations in classroom exercises for the past 4 years. Similar experiments in epidemiology have been done with a more virulent strain of Newcastle disease virus by Andrewes (1), who has shown that a variety of factors presumably not related to aerosol may be involved in the spread of the infection. The limitation of the mild strain of the Newcastle disease virus to the upper respiratory tract of the chick may be dependent on the method of inoculation. Dr. T. Iida, working with Dr. Baetjer, has shown that influenza A virus (the PR8 strain) may also be limited to the upper respiratory tract, at least for the initial phases of the infection, when *small volumes* are inoculated into unanesthetized mice.

Organ Culture and Cell-Virus Relationships

The third part of this discussion will concern this mucosa itself. One method of study has been the preparation of specialized organ cultures of the respiratory epithelia. The technique of growing organized tissues was developed by Honor Fell of the Strangeways Laboratory, who over many years has grown a variety of types of embryonic epithelium for analysis and experiment. For instance, she has shown that normal chick squamous epithelium may be converted to mucous epithelium, even containing some cilia, just by adding a high concentration of vitamin A to the culture medium (7). The obvious question whether this then changes the susceptibility of the epithelium to virus is now under study in our laboratory. We should like to know what happens to the component cells when the mucous epithelium of a chick is exposed to a particular virus. For this reason we grew organ cultures of embryonic trachea and exposed them after 2 days to a concentrated solution of Newcastle disease virus. The numbers of cells which were infected by this exposure over a period of 1 hr was then determined by removing the entire explant after first exposing it to streptomycin and penicillin, then to antibody, and then washing it thoroughly to rid it of any unattached virus or loose cells. The mucosa was then scraped into a solution of trypsin. The cells that were so obtained were washed in the trypsin solution and then inoculated onto a tissue culture plate which allows one to determine the actual number of infected cells.

To our surprise we found repeatedly that instead of a continuous increase in the number of infected cells as the virus stayed on the surface there was a decrease in the number of infected cells. This decrease was not apparent until about 4 hr after the infection had started. We began to suspect that there was something wrong with our technique and for this reason we turned back to study the effect of virus in roller-tube cultures of trypsin-treated chick embryo tissue. As may be seen in Fig. 7, in these preparations there was indeed an increase in the number of infected cells. This is in marked contrast to the almost uniform decrease in the number of infected cells when the virus was placed onto the organ cultures of epithelium (Fig. 8) and when we limited our studies to the mucosa itself.

We then turned to a study of the trachea. The intact tracheas of chicks were used and we found a 100-fold drop occurring in the number of infected cells over a period of 4 or 5 hr (Fig. 9). Obviously the infection became established in the tissue after that time, for when the cultures were followed for a period of 10 to 24 hr, the gradual increase subsequent to this initial drop was easily demonstrated. The explanation of these particular findings seems rather obvious when one compares the reaction of human embryonic tracheal epithelium to influenza virus; there is an apparent sloughing of the cells of the mucosa out into the mucus overlying the mucosa (3). It is well known that the initial phase of this virus infection is limited to a period of some 4 to 8 hr, the time

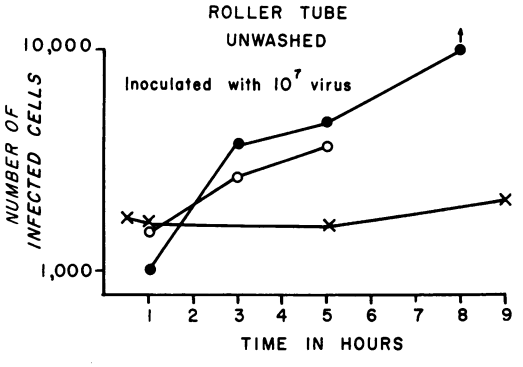

FIG. 7. *Increase in number of infected cells with time of exposure to virus. Cells were treated with antibody and thoroughly washed before titered.*

varying with the dose of virus given and presumably with the multiplicity of the virus infection (Fig. 10).

Our results suggest that the specialized epithelium readily becomes infected during the first hour of exposure and that continued secretion of the mucus into the surface area prevents further infection. Subsequently as the cells become changed by the presence of the virus, they lose their capacity to stick to each other and are pushed out by the epithelium into the mucosa.[1]

In measuring the actual number of infected cells that are present we have limited our studies to the intact epithelium and have washed away the sloughed cells. Many types of epithelium may be studied by this means. We have grown adult human bronchus successfully for a period of 1 to 2 months by the organ culture technique and have studied the reaction of the epithelium to various agents. The cultivation and maintenance of such mucosae is not difficult and offers an excellent method for studying the relationship of specialized epithelial cells to respiratory infections.

We have just begun the study of the relationship of the number of infected cells in the upper respiratory tract to the dose of virus which has been inoculated. To our pleasure it has been possible to take from the chicks which have been infected with the Newcastle disease virus, individual parts of the upper respiratory tract (septum,

[1] More recent experiments on the intact chick indicate that sloughing in the animal itself may not take place during the first phase of virus multiplication (F. B. Bang and M. Foard, *unpublished data*).

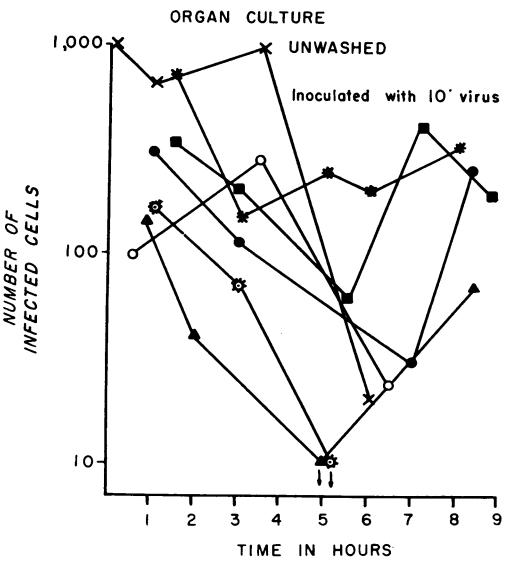

FIG. 8. *Infected cells obtained from organ culture of chick trachea following single exposure to virus. No washing and no culturing after culture exposure.*

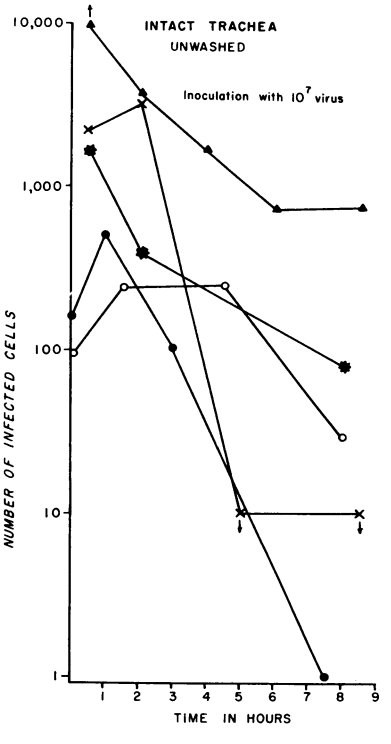

FIG. 9. *Infected cells obtained from trachea immediately after excision from animal. Treatment same as in Fig. 8.*

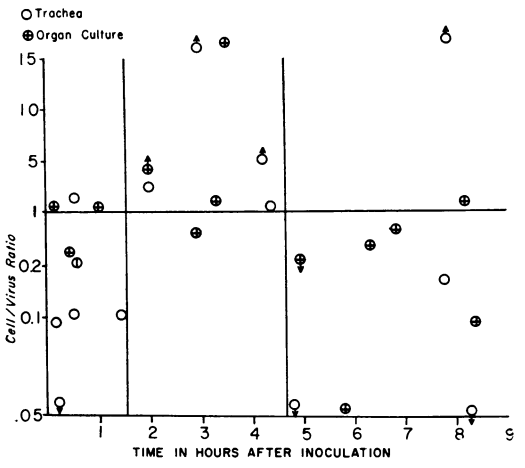

FIG. 10. *Ratio of infected cells to free infectious viruses on organ cultures and entire trachea at intervals after inoculation.*

middle, and posterior conchae) which may be scraped and handled by the same technique as the organ cultures. By this means we have demonstrated the number of infected cells that are present in this area. To our surprise the number of these infected cells seems to be rather small despite the very large dose of perhaps 100 million infectious doses used. This is in contrast to the evidence which has been produced by inoculation of serial dilutions of the virus into the upper respiratory tract. Infection in the latter case may be produced with very dilute solutions of virus, perhaps with as few as ten virus particles. This immediately raises the question: Where does the infection actually take place in the upper respiratory tract? Perhaps our failure to find large numbers of infected cells in the ciliated and mucous epithelium reflects not a low recovery but a direct protective function on the part of this mucosa. It is necessary to determine whether infection in the upper respiratory tract indeed takes place on the mucous-ciliated cells or whether perhaps it takes place on squamous cell, lymphoid, or other areas of the upper respiratory tract which are not directly protected by the mucous blanket.

Summary

Although it has been known for sometime that the activity or function of the mucociliary system in the upper respiratory tract is important in response to the infection, knowledge has rested primarily on a priori grounds, as has been quite properly emphasized by otolaryngologists such as Proetz (18), Crowe (17), and others. It is plainly difficult to study the sequence of events following mucosal infection. The first requirement is comparative study of the gross and mucosal anatomy of the areas so that a minimum of confusion may be introduced when one compares the sinusitis or the coryza of one type of an animal with that of another. Secondly, it is obvious that we need to know not only the direction of flow of the mucociliary blanket in the species of animals concerned but something of the effects of acute and chronic dehydration on those areas. The varities of nonmucous glands which excrete into this area have not been studied adequately as possible mechanisms of producing antibacterial or antiviral substances such as "lysozymes."

In addition to the need for more knowledge of the normal anatomy and physiology, pathogenesis needs study as well. This requires experimental infections which are limited to the upper respiratory tract. Such models may be easier to find than we had formerly thought and may simply depend on the mode of inoculation. If means are found to prevent the inhalation of the virus into the lower respiratory tract, then upper respiratory tract infections may indeed turn out to be amenable to experimental study. Finally, tissue culture techniques have been used for the study of virus-cell relationships extensively, but the variety of tissue cultures that have been studied have been principally on dedifferentiated cells. With the application of the organ culture technique to the study of infection, particularly of the respiratory tract, we have obtained some quantitative idea of the numbers of cells which become infected and the mechanisms whereby the virus spreads from one infected cell to its neighboring uninfected cells.

In each one of these situations, comparative anatomy, physiology, pathogenesis, and the study of cells in organ culture, it is clear that the process of mucous secretion, the consequent formation of a blanket which covers the ciliated cells and is propelled by them in a particular direction, plays a large role in the eventual outcome of the infection.

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